

SAXS-LABDISK: A CENTRIFUGAL MICROFLUIDIC SCREENING PLATFORM FOR PROTEIN STRUCTURE ANALYSIS

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ABSTRACT

A small angle x-ray scattering (SAXS) screening platform for fully automated protein structure analysis based on a centrifugal microfluidic LabDisk is presented. Protein sample (2 μ l), dilution buffer (3 μ l), and screening solution (3 μ l) are split into 40 nl aliquots each (CVs < 5.5%), recombined in predefined ratios to 20 samples of 240 nl and mixed. On-Disk analysis can then be performed in a SAXS beamline. Up to 7 different screenings can be performed in parallel on one disk. For the first time, the SAXS-LabDisk will enable routine SAXS screening of minute protein volumes.

KEYWORDS: Centrifugal microfluidics, Aliquoting, Mixing, Dilution, SAXS

INTRODUCTION

One of the main challenges for structural biology is the visualization of macromolecular structures [1]. SAXS makes it possible to reconstruct such structures directly from solution scattering [2]. This is especially useful for screening of changes in the shape of biological macromolecules due to differences in environmental conditions (pH, salt concentration, ...) [3]. However, for state-of-the-art techniques, consumption of the very expensive protein samples is still at least 6 μ l per condition [4] and the time per measurement is at best 3 min [1]. In most cases this still renders high-throughput multi-parameter screening impractical.

We present a centrifugal microfluidic platform capable of decreasing both, time per measurement and consumed protein volumes in small angle scattering screenings by more than one order of magnitude.

FABRICATION

The disks were designed using AutoCad 2007 (AutoDesk). The PMMA disks (Plexiglas, Evonik, Germany) have a diameter of 115 mm and a thickness of 1 mm. Structures are patterned into both sides of the substrates using CNC micromilling (HSG-IMIT LOAC Foundry Service, Germany). The smallest functional structures on the disk are the geometric valves with a width of 35 μ m and depth of 15 μ m. After structuring the disks are cleaned using isopropanol and sealed from both sides via lamination of adhesive foil (900 320, HJ Bioanalytics, Germany). Finally, the disks are mounted on a custom-built SAXS-LabDisk player (BioFluidix, Germany) for processing (fig. 1).

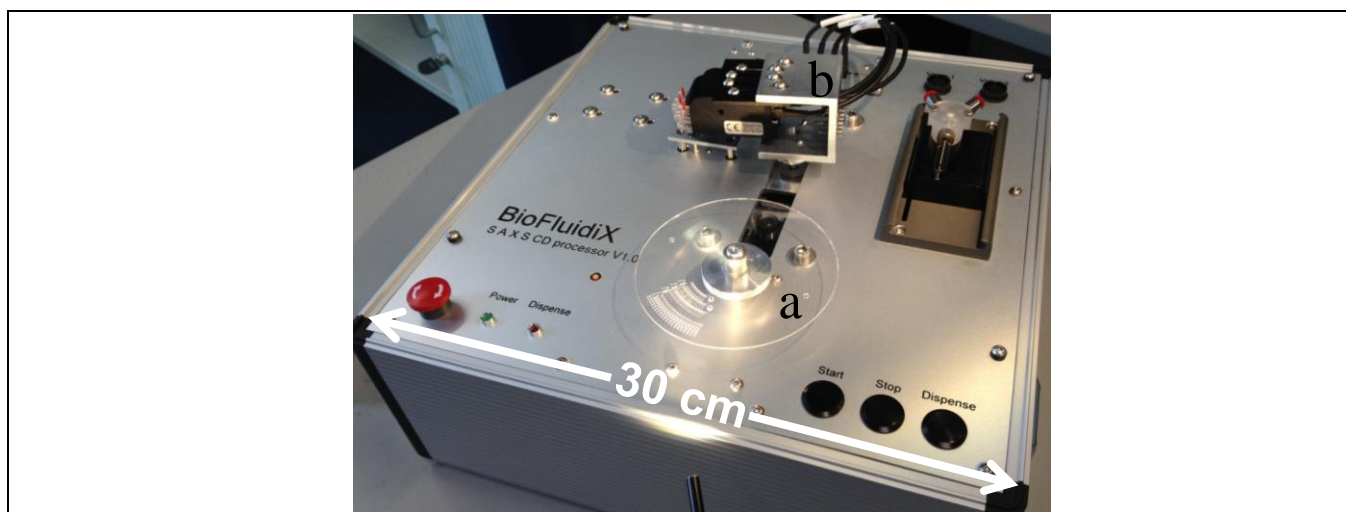


Figure 1: Processing device (custom-built, BioFluidix, Germany) with SAXS-LabDisk (a). The fully automated frequency protocol can be started by the push of one button. For further decrease in volume, below the limit of manual pipettes, Pipe-JetTM dispensers (BioFluidix, Germany) (b) are implemented.

FUNCTIONAL PRINCIPLE AND RESULTS

Protein sample (2 μ l), screening solution (3 μ l) and dilution buffer (3 μ l) are pipetted into the disk (fig. 2). Then, a fully automated centrifugal protocol is started. First, liquids are split into 120 aliquots of 40 nl each. Subsequently, 20 different mixtures are generated by combining six aliquots of the three liquids in varying combinations (fig. 3). The mixtures are then homogenized via reciprocation by an entrapped air-bubble that expands and contracts under oscillating rotational frequencies [5]. Experiments have been performed with four liquids covering a range of typical solutions used in small angle scattering experiments: deionized H₂O, high salt solutions (1M NaCl), high protein solutions (25 mg / ml lysozyme in 1 \times PBS) and highly viscous solutions (33% glycerol v/v in H₂O) (fig. 4).

CONCLUSION AND OUTLOOK

The SAXS-LabDisk is a novel centrifugal microfluidic platform enabling fully automated liquid handling for rapid high-throughput SAXS screening of protein solutions directly at the beam-line. One disk has enough space for seven screenings with 20 measurements each. Including positioning within the beam, the expected total processing time for liquid handling of 7 proteins including SAXS readout of 140 screening conditions is below 15 minutes, compared to > 7 hours with state of the art systems.

The performance of the SAXS-LabDisk for protein structure determination will be evaluated at the beamline PETRA-III at EMBL Hamburg, Germany later this year. The platform is expected to be superior to state of the art in the crucial parameters of time per measurement and sample consumption by more than one order of magnitude.

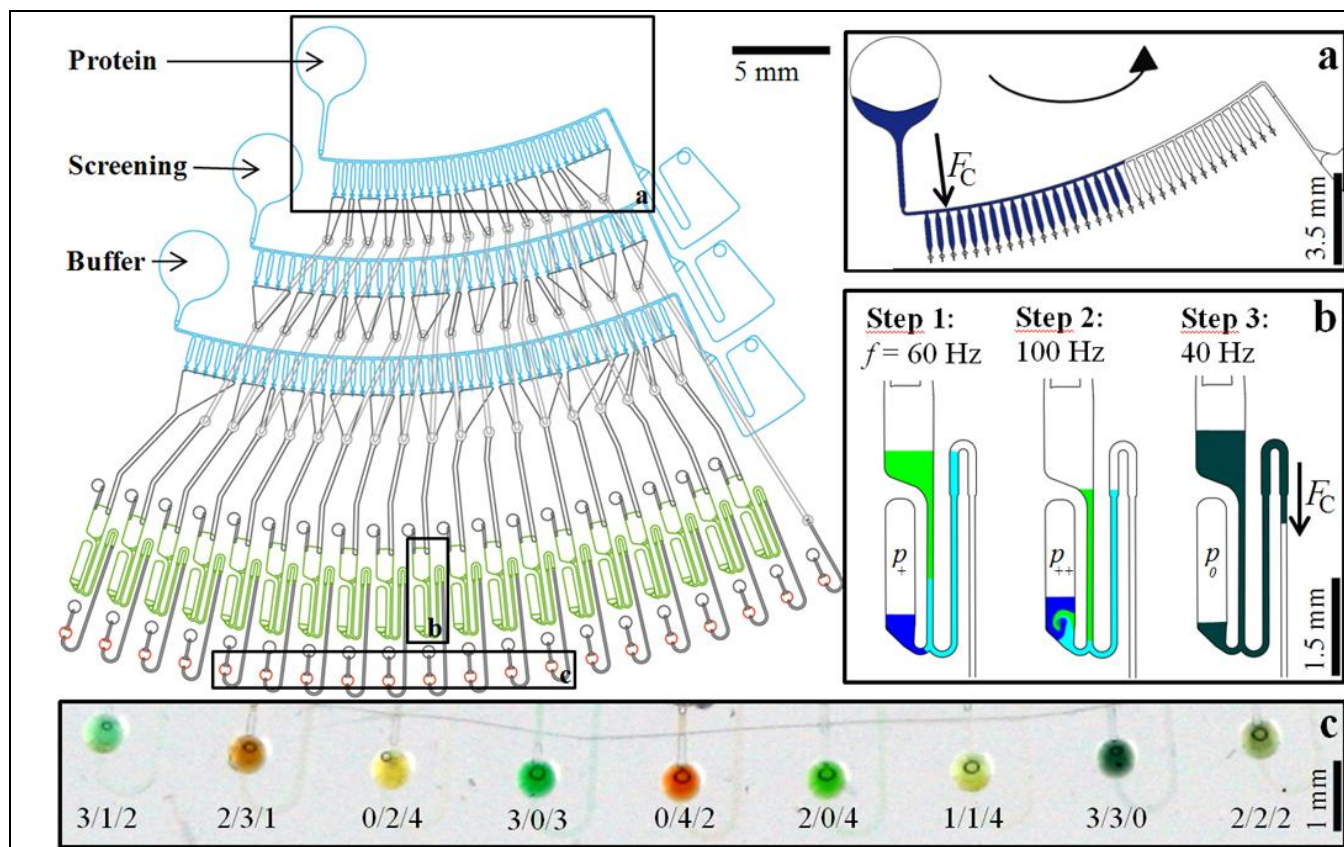


Figure 2: Fluidic principle of SAXS-LabDisk. Inset (a) preparation of 40 nl aliquots. Valving is performed via capillary valves. Inset (b) shows the principle for mixing via repetitive compression and decompression of an enclosed air bubble, due to oscillating centrifugal pressure between different rotational frequencies (Step 1, Step 2). After mixing, the homogeneous solution is transported to the measurement chamber via priming of a siphon due to expansion of the entrapped air at low rotational frequencies (Step 3). Frame (c) shows a photograph of the measurement chambers with microfluidically generated varying protein-/ screening-/ buffer-concentrations. For better illustration blue, red and yellow dyes were added to the protein, screening and buffer inlet.

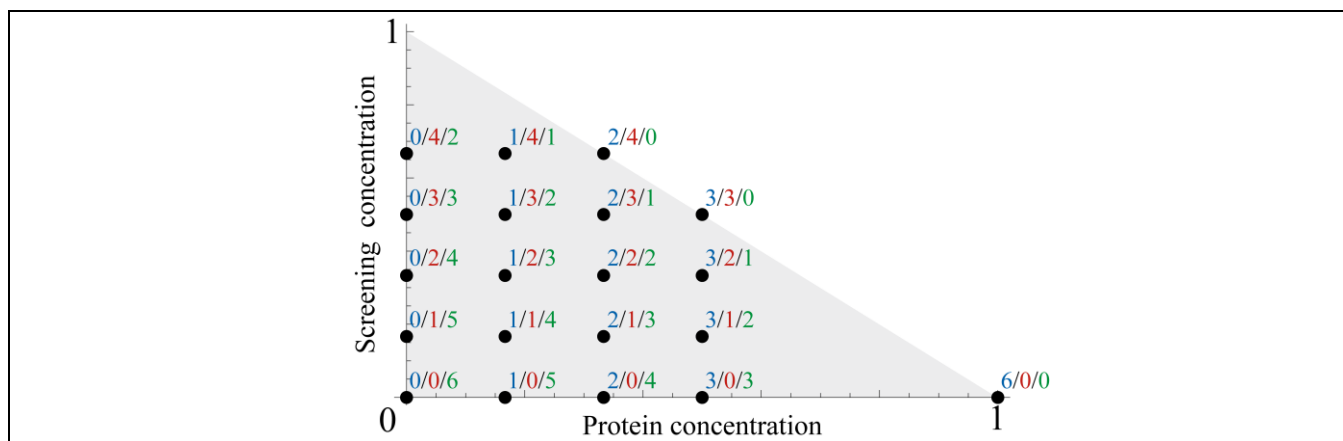


Figure 3: Implemented dilution matrix. Each measurement chamber is represented by one of 20 dots. For each dot the number of protein / screening / buffer aliquots necessary to reach the predefined concentration is given.

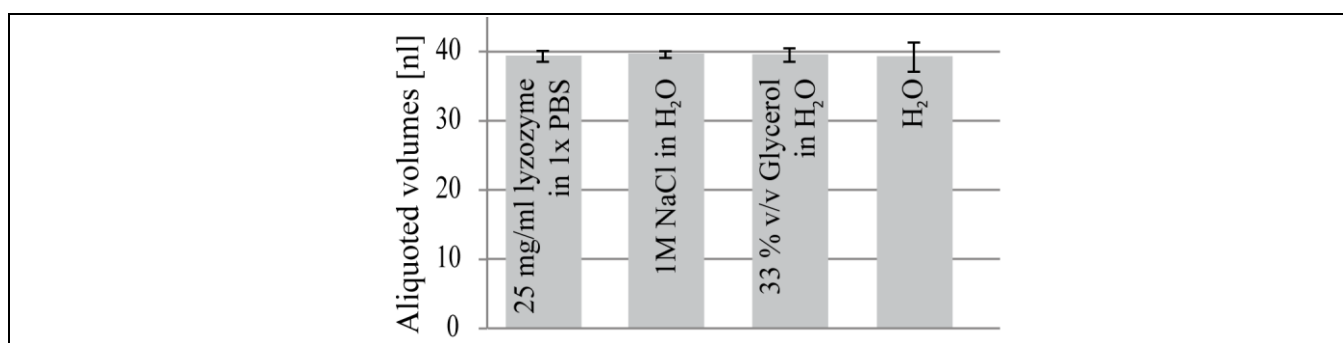


Figure 4: Results for aliquoted liquids at room temperature (100+ measurements each). Liquids with high protein concentration, high salt concentration, high viscosity and deionized H₂O were tested. Error bars represent CVs that are between 1 % and 5.5 %.

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